11/14/80
Minutes

THE DOW CHEMICAL COMPANY

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MIDLAND, MICHIGAN 48640

December 3, 1980

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Attached are the minutes from the November 14 meeting with handouts for your consideration. I have made two corrections on page 2. I will be sending you an agenda for the December meeting shortly.

Sincerely,

John W. Weseloh

John W. Weseloh, Manager
Product Registrations
Health and Environmental Sciences

DEC 1 1. 1980

pjc

Enclosures

INDUSTRY TASK FORCE ON 2,4-D RESEARCH DATA

Meeting of the Technical Committee
NACA Conference Room

Washington, D.C. -- November 14, 1980

9 a.m.

M I N U T E S

TIME/PLACE

The meeting of the Technical Committee of the Industry Task Force on 2,4-D Research Data was held at 9:00 a.m. on November 14, 1980 at the NACA Conference Room, Washington, D.C.

ATTENDANCE

John W. Weseloh (Chairman)	- Dow Chemical Corp.
J. S. Skaptasan	- PBI Gordon Corp.
Donald N. Yoder	- BASF-Wyandotte Corp.
Ronald D. Wilson	- Rhone-Poulenc, Inc.
Don Munger	- Diamond Shamrock
Warren Crummett	- Dow Chemical Corp.
Jim Reid	- Vertac Chemical Corp.
Jim Oliver	- U.S. Department of Agriculture
Kevin Keanney (Part-time)	- Environmental Protection Agency
Patricia Cohn (Part-time)	- Environmental Protection Agency
Phil Kerney (Part-time)	- Environmental Protection Agency
Dudley Thompson	- Environmental Protection Agency

CALL TO ORDER

The meeting of the Technical Committee was called to order at 9:00 a.m. by John Weseloh, Chairman of the Technical Committee. John Conner, Jr. was present as Secretary.

REVIEW OF MINUTES OF TECHNICAL COMMITTEE, OCTOBER 24, 1980

The second line of the last paragraph, page 3, should read "effects of any . . ." Except for the addition of the word "of" noted correction, the Committee approved the October 24, 1980 minutes.

DR. WARREN CRUMMETT

John Weseloh introduced Dr. Warren Crummett from Dow. Dr. Crummett attended and reported on the 2,4-D workshop in Rome. He summarized the following areas discussed at the Rome ~~2,4-D~~ workshop: analytical; environmental fate and levels; inceneration; biochemical and animal toxicology, and observations in man.

Dr. Crummett distributed a paper authored by W. P. Cochrane, et al. of Canada entitled "Analysis of Technical and Formulated Products of 2,4-Dichlorophenoxy Acetic Acid for the Presence of Chlorinated Dibenzo-P-Dioxins." (Attachment 1)

Dr. Crummett reported that there are no validated analytical methodologies for dioxins. Analytical equipment and methods will vary from company to company.

VALIDATED ANALYTICAL METHODOLOGY

The Committee was of the opinion that there is a need to develop validated analytical methods for the analysis of 2,4-D products for the presence of dioxins. To this end, Dr. Crummett distributed a paper entitled "Estimate of Time Required to Develop Methods for the Analysis of 2,4-D Products for the Presence of Dioxins." (Attachment 2)

The Committee was of the opinion that the two chronic, teratology and reproduction studies required by EPA must be conducted with a material for which a validated analytical method had been developed. Four potential labs were identified by the Committee, which could develop validated analytical methods: University of Nebraska, Wright State, Research Triangle Park (EPA), and Dow Chemical Company.

The Committee decided that it would recommend to the Task Force that the Task Force sponsor the development of a good validated method.

CANADA

Because of recent developments in Canada and the country's concern over reported dioxin in 2,4-D, the Technical Committee recommended that a Canadian regulatory official be invited to participate on the Technical Committee.

P. KERNEY AND J. OLIVER - 2,4-D STUDIES

P. Kerney and J. Oliver reported on joint EPA/USDA exposure and impurity studies on 2,4-D. The exposure study measured exposure to 2,4-D by 26 ground applicators and 17 aerial applicators. The study should be completed by December 1, 1980.

J. Oliver distributed attachment 3 which tentatively identified impurities found by him in certain 2,4-D products. Dr. Oliver discussed his analytical methodology and emphasized that his work was exploratory and preliminary.

PATRICIA COHN

Ms. Cohn of EPA advised the Technical Committee that EPA would soon issue a notice clarifying and deferring certain acute data requirements for formulators. Ms. Cohn then explained that the Enforcement Division of EPA would collect 2,4-D samples for analysis.

The Technical Committee discussed with Ms. Cohn its concern that the first order of business should be to complete analytical work on 2,4-D before beginning chronic testing.

After receiving clearance from EPA for any conflict of interests, the Technical Committee will investigate Wright State and the University of Nebraska for the performance of the necessary analytical work. This investigation will be conducted by Jim Reid and Don Munger of the Technical Committee.

TESTING SAMPLES

Each member of the Task Force will request 200 Kgs. of 2,4-D, representative of production, for testing and sampling. J. Weseloh distributed to attendees a draft of the Memorandum of Confidentiality prepared by counsel for review and comment. (Attachment 4). J. Weseloh pointed out to Ms. Cohn that the decision had been made to test a single representative sample of technical 2,4-D. For the purpose of determining a representative content of 2,4-D in Task Force products, members of Task Force will submit to counsel copies of 2,4-D acid labels. These labels, in turn, will be reviewed by members of the Technical Committee at its next meeting.

Pat Cohn advised the Committee that the Agency was deferring formulators from certain acute testing.

EPA will advise the Technical Committee whether manufacturing use products or technical products are to be tested.

R. Wilson distributed copies of handout 5 (attachment 5) "Protocol for a Chronic Feeding-Oncogenicity Study on 2,4-D Administered in the Diet to CDF Fisher 344 Rats." R. Wilson discussed the protocol and bid system including site visits. Each member of the Task Force should review handout 5 as soon as possible and comment on the same.

NEXT MEETING

The next meeting of the Technical Committee will be on December 17, 1980 at the NACA Conference Room. It is anticipated that the meeting will last half a day, beginning at 9 a.m.

ADJOURNMENT

The meeting was adjourned at 2:30 p.m.

John D. Conner Jr.

JOHN D. CONNER, JR.

(Attachment 1)

Handout 1

ANALYSIS OF TECHNICAL AND FORMULATED PRODUCTS OF 2,4-DICHLOROPHENOXY ACETIC ACID FOR THE PRESENCE OF CHLORINATED DIBENZO-P-DIOXINS

W.P. Cochrane, J. Singh, W. Miles, B. Wakeford, J. Scott

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Presented at the Workshop on the "Impact of Chlorinated Dioxins and Related Compounds on the Environment" October 22,24, 1980, Rome (Italy).

To be published in a symposium series by Pergamon Press, Oxford.

ABSTRACT

Sixteen samples of 2,4-D ester and amine, both technical and formulated products, representing current Canadian supplies, were analysed for the presence of different chlorinated dibenzo-p-dioxins. The method of analysis involved extraction, partitioning, multiple column chromatography with final quantitation being performed by gas chromatography/mass spectrometry using a packed column. Isomer identification was achieved on a capillary column while dioxin identity was confirmed using high resolution mass spectrometry. The methodology provided for recoveries in excess of 85% with the limit of detection being 1 ppb. Eight out of nine esters and four out of seven amine samples were found to contain di-, tri- and tetra-chlorodibenzo-p-dioxins. Ester formulations showed significantly higher levels of contamination than the amine formulations. The tetra-chlorodioxin observed was the 1,3,6,8-isomer as verified by the synthesis of an authentic analytical standard.

In addition ten 2,4-D technical acid samples from 4 different sources did not contain any mono-, di-, tri- or tetra-chlorodioxins above the 1 ppb detection limit, although low levels of polychlorinated biphenyl ethers were observed.

KEYWORDS

Gas chromatography-mass spectrometry, chlorinated dioxins, 2,4-D formulations, TCDD isomers.

INTRODUCTION

There are 75 possible isomers in the class of compounds known as the polychlorodibenzo-p-dioxins (PCDDs). PCDDs together with the polychlorinated dibenzo-furans (PCDFs) and polychlorinated diphenyl ethers (PCDPEs) have been found as microcontaminants in commercial chlorophenols (Nilsson and Renberg, 1974; Jensen and Renberg, 1972; Firestone and co-workers, 1972; Rappe, Gava and Buser, 1978b) and in pesticides which use chlorophenols in the manufacturing process. While the hexa-, hepta- and octa-chlorodibenzo-p-dioxin isomers have been reported by various workers (Woolson, Thomas and Ensor, 1972; Villanueva and co-workers, 1973; Johnson and co-workers, 1973) in trichlorophenols, tetrachlorophenols and pentachlorophenol (PCP) at levels ranging from the low ppb (i.e., HxCCD) to 1000 ppm (OCCD), the highly toxic 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) has not been found in commercial PCP (U.S. EPA, 1978) since the appropriate precursors are not present (Johnson and co-workers, 1973). However, trace amounts of 2,3,7,8-TCDD have been found in 2,4,5-TCP (Firestone and co-workers, 1972; Elvidge, 1971) at levels ranging from 90 ppb to 6.2 ppm, in addition to ppm levels of 2,7-dichlorodibenzo-p-dioxin, 1,3,6,8-TCDD and pentachlorodioxins. Also, 2,3,7,8-TCDD can be carried through into products made from 2,4,5-TCP, such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Elvidge, 1971), hexachlorophene and mixed 2,4-D/2,4,5-T formulations (1:1 mixture of butyl esters known as Agent Orange on Herbicide Orange). Herbicide Orange was found to contain 0.1-47

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ppm 2,3,7,8-TCDD together with smaller amounts of other less toxic PCDDs and PCDFs (Rappe and co-workers, 1978a). Similarly, condensation of 2,4,6-trichlorophenate during its manufacture under alkaline conditions produces the expected 1,3,6,8-TCDD, as well as the isomeric 1,3,7,9-TCDD by Smiles rearrangement (Leng, 1979). Recent reviews have summarized the current situation with respect to dioxin analysis of PCP, 2,4,5-T and the polychlorinated biphenyls (Firestone, 1977; Rappe and co-workers, 1979). In the area of the lower chlorinated phenols and phenoxyacetic acids, no PCDDs were detected in 2,4- and 2,6-dichlorophenols (Firestone and co-workers, 1972). Woolson found HxCDD in 1 sample of 2,4-D at a level between 0.5 and 10 ppm. Only tetra-, hexa-, hepta- and octachlorodioxin results were reported and none were observed in 23 other 2,4-D samples, as well as, three 2,4-DB and two 2,4-DP samples. Similarly, no di- to hexa-CDD isomers were found in older Scandinavian formulations of 2,4-D although one 2,4-D sample did contain 0.06 ppm tetra-CDF (Norstrom and co-workers, 1979). However, the analysis of 2 Herbicide Orange samples showed the presence of 1,3,6,8-TCDD (in addition to the expected 2,3,7,8-isomer) and 1,3,7-tri-CDD which were postulated as being formed from condensation products of 2,4-di and 2,4,6-trichlorophenols (Rappe and co-workers, 1978a).

As part of a continuing program concerning the identification and quantitation of microcontaminants in pesticide formulations, this paper reports the results of dioxin analysis of 2,4-D amine, esters and acids.

EXPERIMENTAL

Sample Preparation

2,4-D ester. Transfer 2.0 g sample onto a silica gel column (50 g of Merck Keiselgel 60 activated overnight at 125°C. Elute with 150 mL of 30% CH_2Cl_2/hexane, discard first 30 mL and collect remainder in a 250 mL RB flask. Concentrate to 1-2 mL and proceed to alumina column clean-up.

2,4-D acid. Dissolve 10.0 g sample in 400 mL of 1:1 acetonitrile/water with 10% methanol added. Partition three times with 100 mL hexane. Combine the organic layers and wash twice with 50% methanol/water. Dry and concentrate the extract to 1-2 mL and proceed to the alumina column clean-up.

2,4-D amine. Dissolve 2.0 g sample in 100 mL water and partition three times with 25 mL hexane. Use iso-propanol to break any emulsions. Combine organic layers, wash twice with 20 mL water, dry and concentrate to 1-2 mL and proceed to the alumina column clean-up.

Alumina column clean-up. Transfer sample extracts onto alumina column (15 g Woelm Basic Alumina Activity I). Elute with 100 mL hexane and 100 mL 2% CH_2Cl_2/hexane, discard. Collect the next 100 mL 5% CH_2Cl_2/hexane and 100 mL 10% CH_2Cl_2/hexane. Concentrate to 1-2 mL and transfer to a 5 mL graduated centrifuge tube. Evaporate to dryness under a gentle stream of nitrogen. Dissolve residue in appropriate volume of hexane.

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GC-MS analysis

A 25 m fused silica capillary column coated with SP 2100 (Hewlett Packard No. 19091-60025) was coupled to a Finnigan 4000 quadrupole mass spectrometer equipped with the INCOS data system. Analysis were performed isothermally at 225°C using the splitless injection of samples at a 20 p.s.i. column pressure. PCDDs were monitored using specific masses - m/e 218 and 220 for isomers of monochloro-, 252 and 254 for dichloro; 286 and 288 for tri-chloro- and 320 and 322 for tetrachlorodioxins. Specific isomers were identified by comparing their column retention with reference compounds and confirmed by high resolution mass spectrometry analysis via elemental composition and fragmentation patterns. The lower limit of detection was 1 ppb.

For high-resolution GC/MS analysis, the gas chromatograph was equipped with a 6 ft. x 1/4 in. glass column packed with either 3% SE-30 ultra-phase on Chrom 750 (80-100 mesh) or 3% OV-17 on Chrom 750 which was coupled to a Kratos MS-50 mass spectrometer by means of a single stage glass jet separator. Single ion monitoring was carried out at m/e 321.8936 at a resolution of 20,000 (10% valley) for tetra-chlorodioxins. Source temperature 250°C, separator 300°C, emission current 500 uA, accelerating voltage 8.0 Kv, detection limit 20 x 10^{-12} g. Full scan data was acquired at 10,000 resolution using the INCOS data system. The major peaks of the isotopic chlorine cluster were all within ± 10 ppm of the correct mass value.

RESULTS AND DISCUSSION

The levels of PCDDs found in the 2,4-D esters and amine samples analysed are shown in Table 1, and represent current Canadian supplies of these products. Since these formulations contained varying amounts of active ingredient the quantitation of the PCDDs was based on the guaranteed 2,4-D acid equivalent for easier comparison of results. The six 2,4-D iso-octyl esters showed levels of 2,7- or 2,8- dichlorodioxin ranging from 104 ppb to 4.2 ppm. These di-CDD isomers are the expected dimerization products of the intermediate 2,4-dichlorophenol used in the 2,4-D manufacturing process (Leng, 1979). Levels of tri-CDD varied from 346 ppb to 2.0 ppm while the presence of tetra-CDD ranged from 226 ppb to 1.7 ppm. The tetra-CDD present in the 2,4-D samples displayed a 0.80 retention time relative to the 2,3,7,8-TCDD isomer on the 3% SE-30 packed column. It was identified as the 1,3,6,8-isomer via capillary column and high resolution fragmentation comparisons with an authentic standard. On capillary column two peaks were resolved, i.e. 1,3,6,8- and 1,3,7,9-TCDD, the normal and Smiles-rearranged dimerization products of 2,4,6-trichlorophenol.

The tri-CDD in all samples appeared earlier than the 1,2,4-isomer, used as an initial standard, and resulted in baseline separation between the two on capillary column. Since the 2,7- (or 2,8)-diCDD and 1,3,6,8-/1,3,7,9-tetra CDDs had been identified, it was found that the cross condensation product of 2,4-dichlorophenol with 2,4,6-trichlorophenol gave a single tri-CDD peak with the same retention time as that found in the sample on both packed and capillary columns. Therefore, this tri-CDD is probably the 1,3,7- or 1,3,8-

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isomer as expected from the above condensation reaction. A comparison of the high resolution mass spectra of the synthesised tri-CDD and that found in the samples were identical. The mass spectral difference between the 3:0 (i.e. 1,2,4-isomer) and the 2:1 (i.e. 1,3,7-isomer) ring chlorine substitution becomes very apparent in the m/e 50-150 range (Buser and Rappe, 1978). Recoveries of di-, tri- and tetra-CDDs from spiked samples was greater than 85% when added in the 1-1000 ppb range.

TABLE 1 Levels of PCDDs in 2,4-D Technical Materials and Formulations

Sample	Type	Percent 2,4-D Acid Equivalent	PCDDS (ppb) Di-	Tri-	1,3,6,8-Tetra
1	Iso-octyl ester	65.4	**[a]	346	226
2	"	65.4	2722	2079	717
3	"	50	4200	1632	1752
4	"	65.1	104	639	315
5	"	60	1238	1825	852
6	"	70	109	929	486
7	Mixed butyl ester	Technical	102	684	317
8	"	50	-	-	-
9	"	Technical	200	160	210
10	Dimethylamine salt	50	-	38	54
11	"	50	-	584	278
12	"	50	5	54	20
13	"	50	-	-	-
14	"	60	33	533	208
15	"	50	-	-	-
16	"	50	-	-	-

[a] Level not reported due to co-eluting interferences.

While all 2,4-D iso-octyl ester samples contained PCDD contaminants, only two of the three 2,4-D mixed butyl esters and four of the seven 2,4-D amines showed the presence of chlorodioxins (Table 1). Also the esters products showed significantly higher levels of contamination than the amine formulations. In addition, ten 2,4-D technical acid samples from 4 different manufacturing sources were analysed for mono-, di-, tri- and tetra-CDDs down to the 1 ppb level and hexa-CDD at the 10 ppb level and none were found. However, the presence of low levels of dichloro-, trichloro- and tetrachloro-diphenyl ether isomers were observed in all cases.

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The results reported in this study differ from those found for Scandinavian formulations of 2,4-D (Norstrom and co-workers, 1979). Although significant variations exist between individual samples, other contributing factors may be cross-contamination, the source of starting material and/or the process used in the manufacture of the 2,4-D esters and amines.

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(Attachment 2) Handout 2

ESTIMATE OF TIME REQUIRED TO DEVELOP METHODS FOR THE ANALYSIS OF 2,4-D PRODUCTS FOR THE PRESENCE OF DIOXINS

Estimates are given for the amount of work required for development of two types of methods for the analysis of 2,4-D products for the presence of dioxins. The estimates given are the time required for skilled workers with a background in this type of synthesis and analysis. Someone unfamiliar with the techniques might require up to three times longer to carry out the synthesis and method development.

One of the estimates is for a method which would detect all isomers of di-, tri-, tetra-, hexa-, hepta-, and octachlorodibenzo-p-dioxin but would not separate many of the isomers so results would be reported as dichlorodibenzo-p-dioxin, trichlorodibenzo-p-dioxin, etc., except that a separate result would be calculated for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). (This 2,3,7,8-TCDD result would not be isomer specific). To be certain that all isomers of each dioxin would be detected, if present, retention time data on all isomers would be needed. Such data is available for the tetra- and hexachlorodibenzo-p-dioxin isomers (Anal. Chem. 52, 2045 (1980)). A few micrograms of the unavailable di- and trichlorodibenzo-p-dioxins would have to be synthesized along with milligram amounts of 1,3,7-trichlorodibenzo-p-dioxin. Quantitation of dichlorodibenzo-p-dioxins would be based on 2,7-di-, quantitation of trichlorodibenzo-p-dioxins would be based on 1,3,7-tri-, quantitation of tetrachlorodibenzo-p-dioxins (except 2,3,7,8-TCDD) would be based on 1,3,6,8-TCDD, and quantitation of hexa-, hepta-, and octachlorodibenzo-p-dioxin would be based on commercially available isomers.

An estimate is also given for development of an isomer specific method for detecting all di-, tri-, tetra-, hexa-, heptachlorodibenzo-p-dioxins, and octachlorodibenzo-p-dioxin in 2,4-D products. Synthesis requirements are about the same as for the other method when quantitation is based on 2,7-di, 1,3,7-tri-, and 1,3,6,8-tetra-. Estimated method development time is also the same because isomer specific techniques have been published.

ISOMER SPECIFIC ANALYSIS

	Estimated Time
Prepare and Validate Standards (Weighable quantities of critical standards, few micrograms of non-critical isomers)	8 man-weeks[a,e]
Method Development Develop separations of dioxins from products Extend method in Anal. Chem. 52, 2045 (1980) to di- and trichlorodioxins	8 man-weeks[a,b,f]
Validate Methods for the Individual Products	
Recovery data on all isomers	4 weeks per product
Most likely compounds and highers	2 weeks per product
Analysis Time Per Sample Using Validated Method	
Several samples at one time	12 man-hours per sample[d]
Analysis of only one sample	30 man-hours

a,b,d Footnotes with Non Isomer Specific Estimate

e Preparation and characterization of milligram quantities of all di-, tri-, tetra-, hexa-, and heptachlorodibenzo-p-dioxins would require several more man-months.

f Development time would be about the same for non isomer specific and isomer specific analysis since isomer specific techniques have been developed (Anal. Chem. 52, 2045 (1980)). Sample analysis time for non isomer specific analysis would be shorter and time could be reduced further if the analysis was only for detection of the most likely dioxins (including higher chlorinated dioxins).

NON ISOMER SPECIFIC ANALYSIS WITH SEPARATE 2,3,7,8-TCDD DETE

Prepare and Validate Standards

Method Development

Develop separation and cleanup of
sufficient sensitivity (down to 1 ppb)

Validate Methods for Individual Products

Analysis Time Per Sample Using Validated Method

Several samples at one time

Analysis of only one sample

a Estimate for Dow or others with background in dioxin pre
analysis. Without this background, the estimate should

b Estimate for method development for the various esters.
will require additional time.

c Existing procedures could be adapted in a shorter time,
separation techniques should be improved, if possible, a
should be tried with 2,4-D products from various commerc

d Estimate is man-hours. Elapsed time would be three days
assumes equipment is already set up.

COMMENTS ON METHOD DEVELOPMENT

The present validated methods for TCDD in 2,4,5-T esters use 20% benzene hexane to separate the TCDD from the ester. Benzene should be eliminated some development time should be spent on other methods of separation.

The extent of interferences that will be present when determining dioxins 2,4-D esters is not known. To have a universal method, the cleanup may r use of reverse-phase LC for even the non isomer specific method. This ty cleanup increases GC-MS time because more fractions are obtained.

(Attachment 3)

Handout 3
Jim Oliver

The compounds whose structures are shown on the following pages have been tenatively identified as impurities in certain 2,4-D products. This is strictly preliminary information and should not be distributed or quoted at this time because

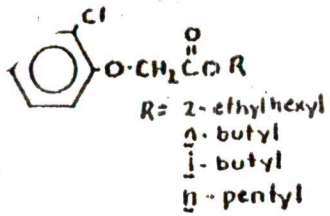
a. some assignments have not yet been confirmed by comparing unknowns with authentic samples.

b. levels of the impurities have not been quantitated.

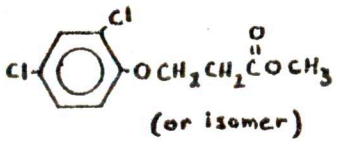
c. some of the impurities have been thus far detected in only one product; others are common to several products.

d. controls have not been adequately analyzed.

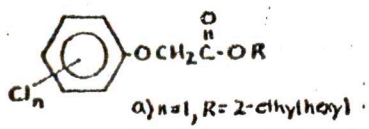
A preliminary report of the investigation will be presented at the American Chemical Society Spring Meeting in Atlanta, March, 1981.



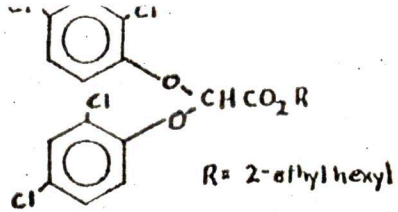
R= 2-ethylhexyl
n-butyl
i-butyl
n-pentyl



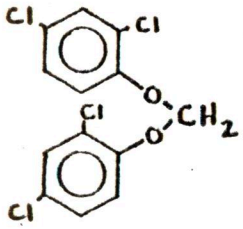
(or isomer)

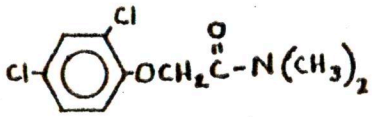


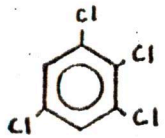
a) n=1, R= 2-ethylhexyl
b) n=3, R= 2-ethylhexyl

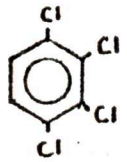


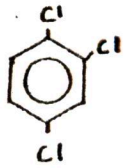
R= 2-ethylhexyl

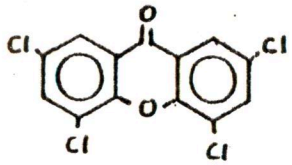


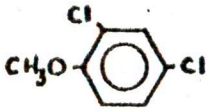


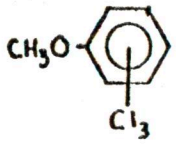


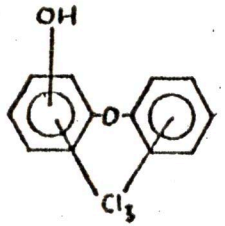


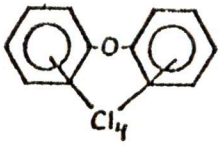


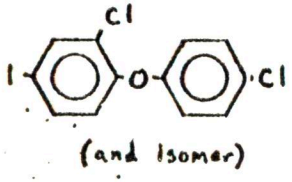




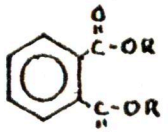




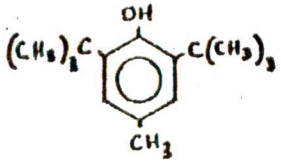


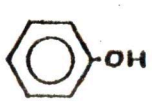


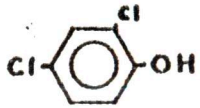
(and isomer)

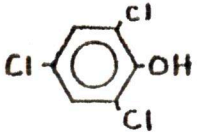


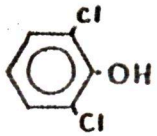
R= 2-ethylhexyl

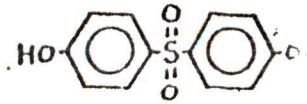


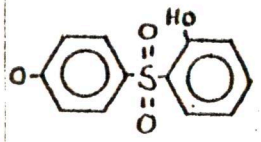


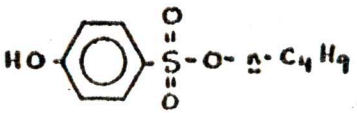


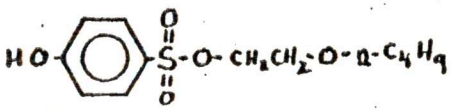


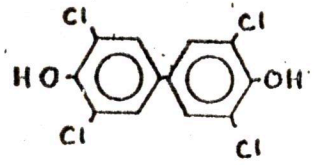


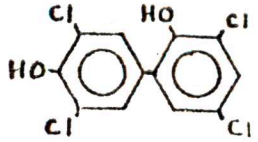


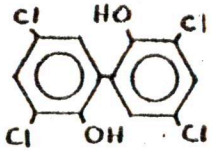


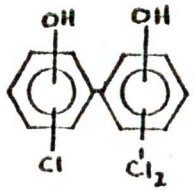


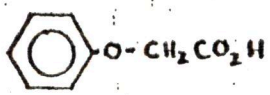


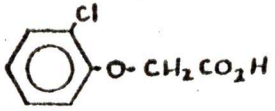


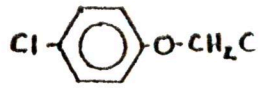


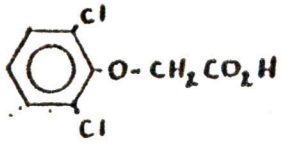


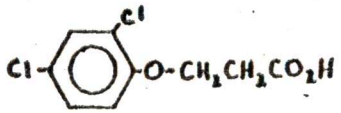




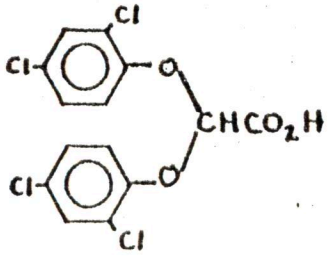


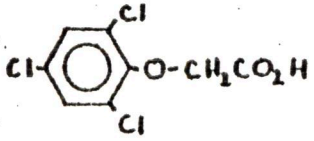


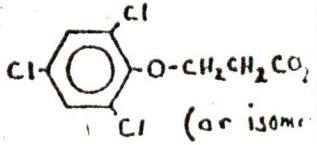




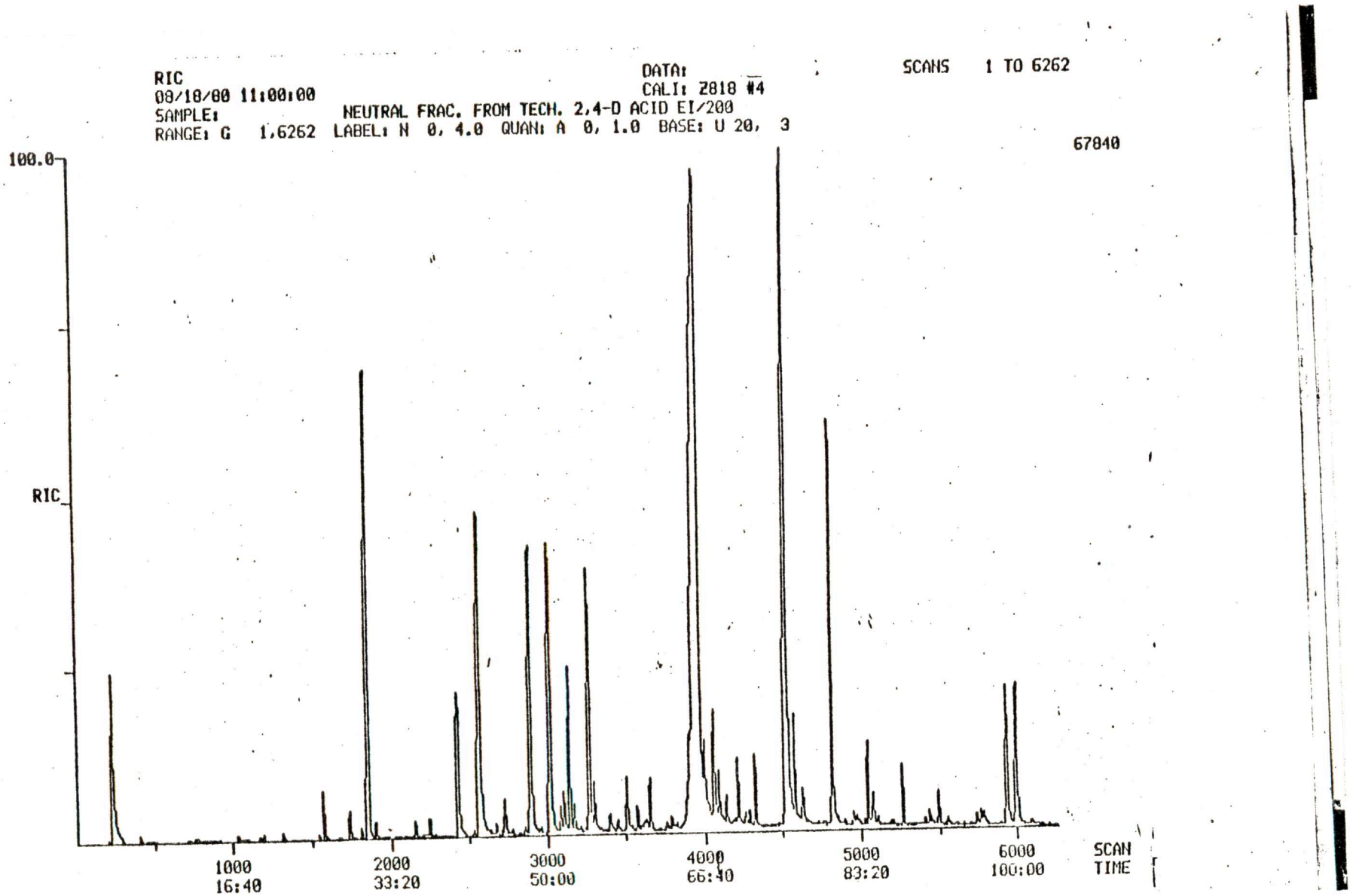
(or isomer)







(or isome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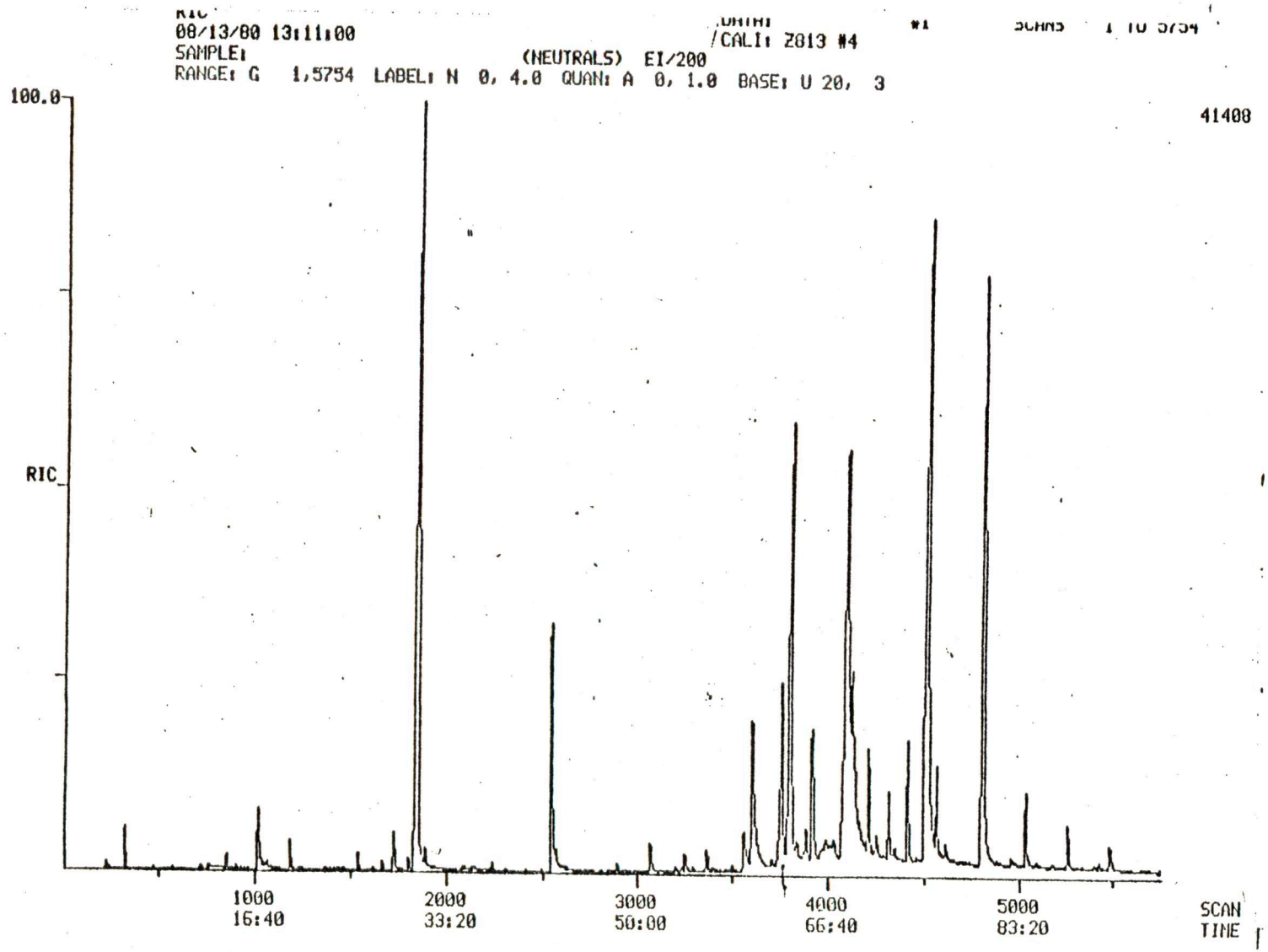
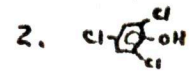
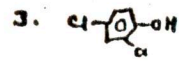
SAMPLE:
RANGE: G 1.6262 LABEL: N 0, 4.0 QUAN: A 0, 1.0 BASE: U 20, 3
NEUTRAL FRAC. FROM TECH. 2,4-D ACID EI/200
DATA:
CALI: Z818 #4
SCANS 1 TO 6262
67040
100.0
RIC
1000
16:40
2000
33:20
3000
50:00
4000
66:40
5000
83:20
6000
100:00
SCAN
TIME

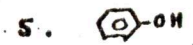

RIC
08/13/00 13:11:00
SAMPLE:
(NEUTRALS) EI/200
/CALI: Z813 #4
RANGE: G 1,5754 LABEL: N 0, 4.0 QUAN: A 0, 1.0 BASE: U 20, 3
100.0
41400
RIC
1000
16:40
2000
33:20
3000
50:00
4000
66:40
5000
83:20
SCAN
TIME

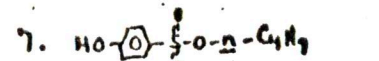
HPLC of a phenol mixture from a butoxyethyl ester

* silica column, 1CV : cyclohexane + 0.1% HOAc
2CV : 4:1 (v:v) DCM:THF
10% → 60% / 10 min, 3 mL/min

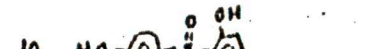
2. 

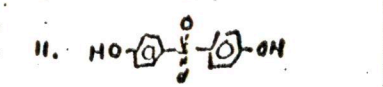
3. 

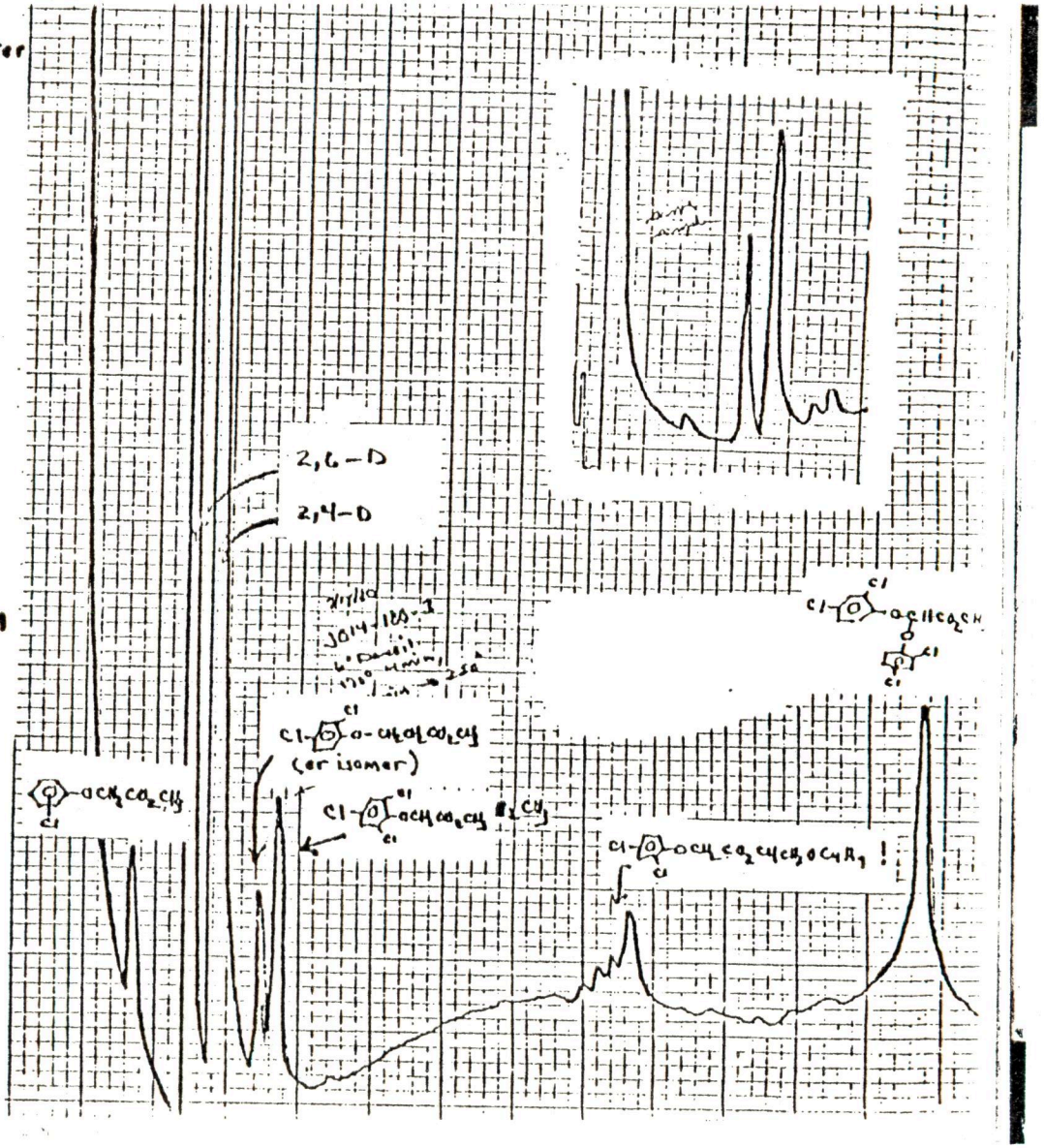
5. 

7. 

9. 

10. 

11. 



(Attachment 3)

Handout 3
Jim Oliver

The compounds whose structures are shown on the following pages have been tenatively identified as impurities in certain 2,4-D products. This is strictly preliminary information and should not be distributed or quoted at this time because

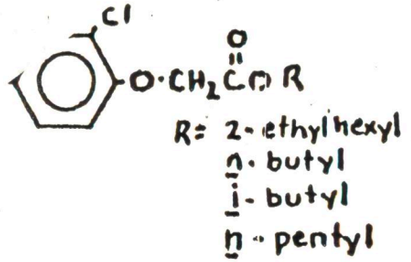
a. some assignments have not yet been confirmed by comparing unknowns with authentic samples.

b. levels of the impurities have not been quantitated.

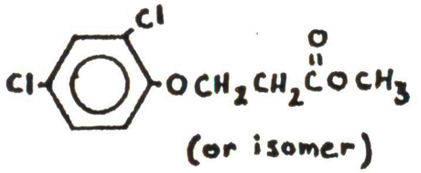
c. some of the impurities have been thus far detected in only one product; others are common to several products.

d. controls have not been adequately analyzed.

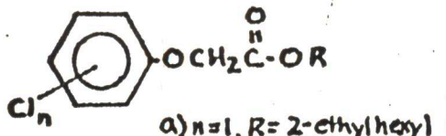
A preliminary report of the investigation will be presented at the American Chemical Society Spring Meeting in Atlanta, March, 1981.



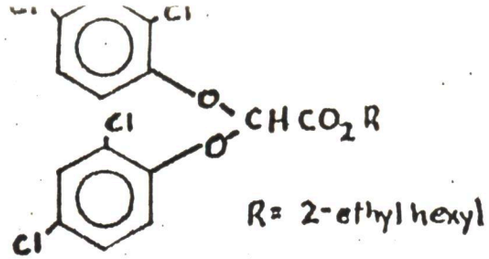
R= 2-ethylhexyl
n-butyl
i-butyl
n-pentyl



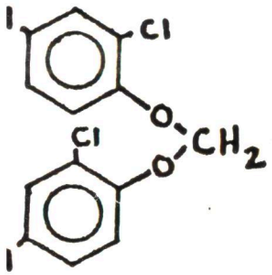
(or isomer)

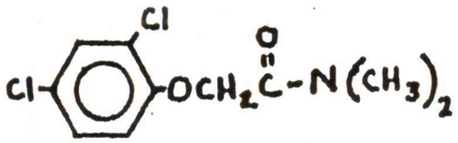


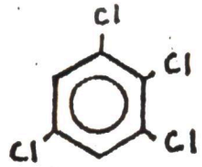
a) n=1, R= 2-ethylhexyl
b) n=3, R= 2-ethylhexyl

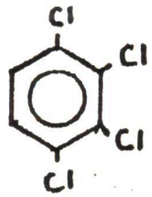


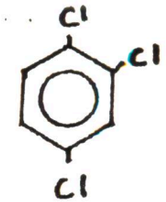
R= 2-ethylhexyl

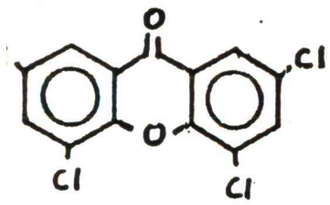


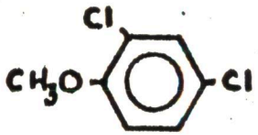


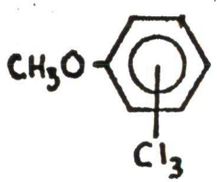


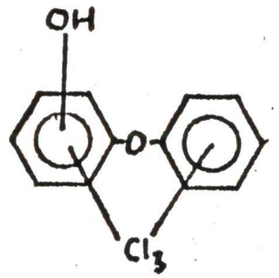


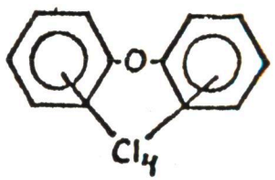


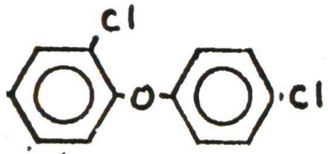












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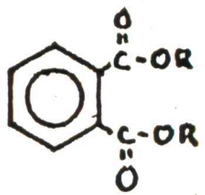

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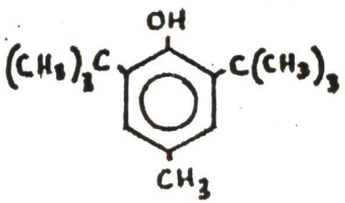

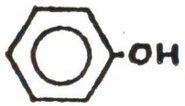

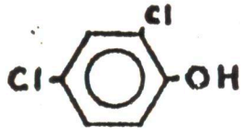

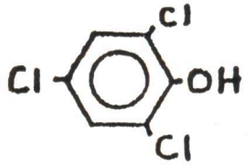

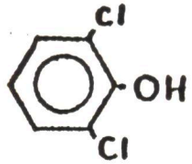

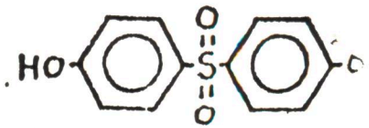

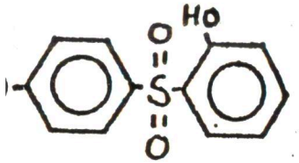

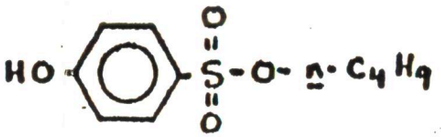

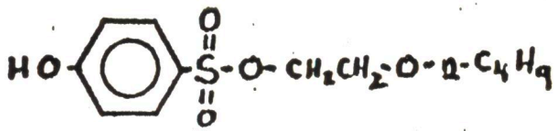

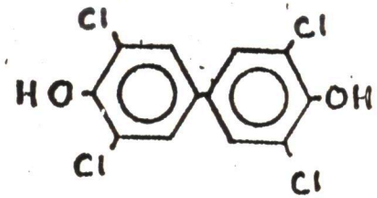

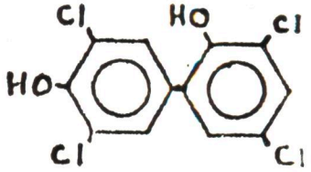

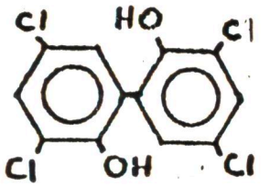

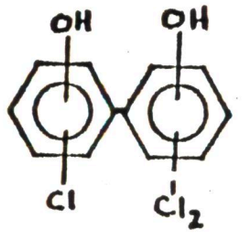

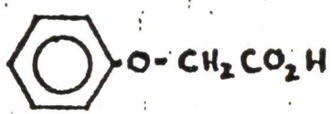

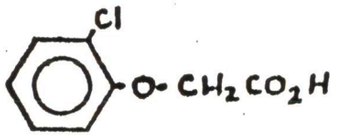

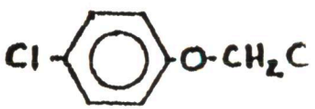

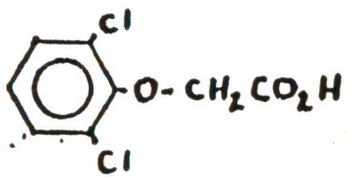

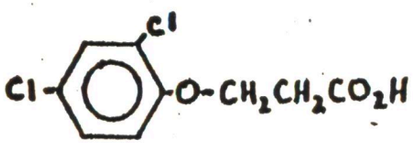

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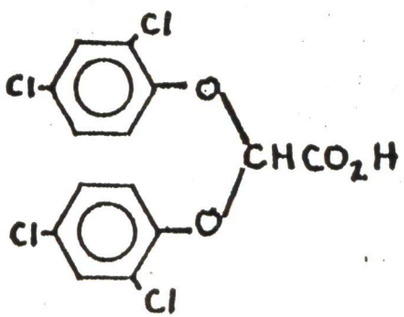

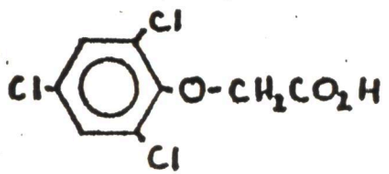

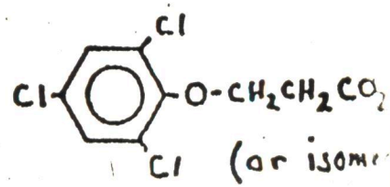

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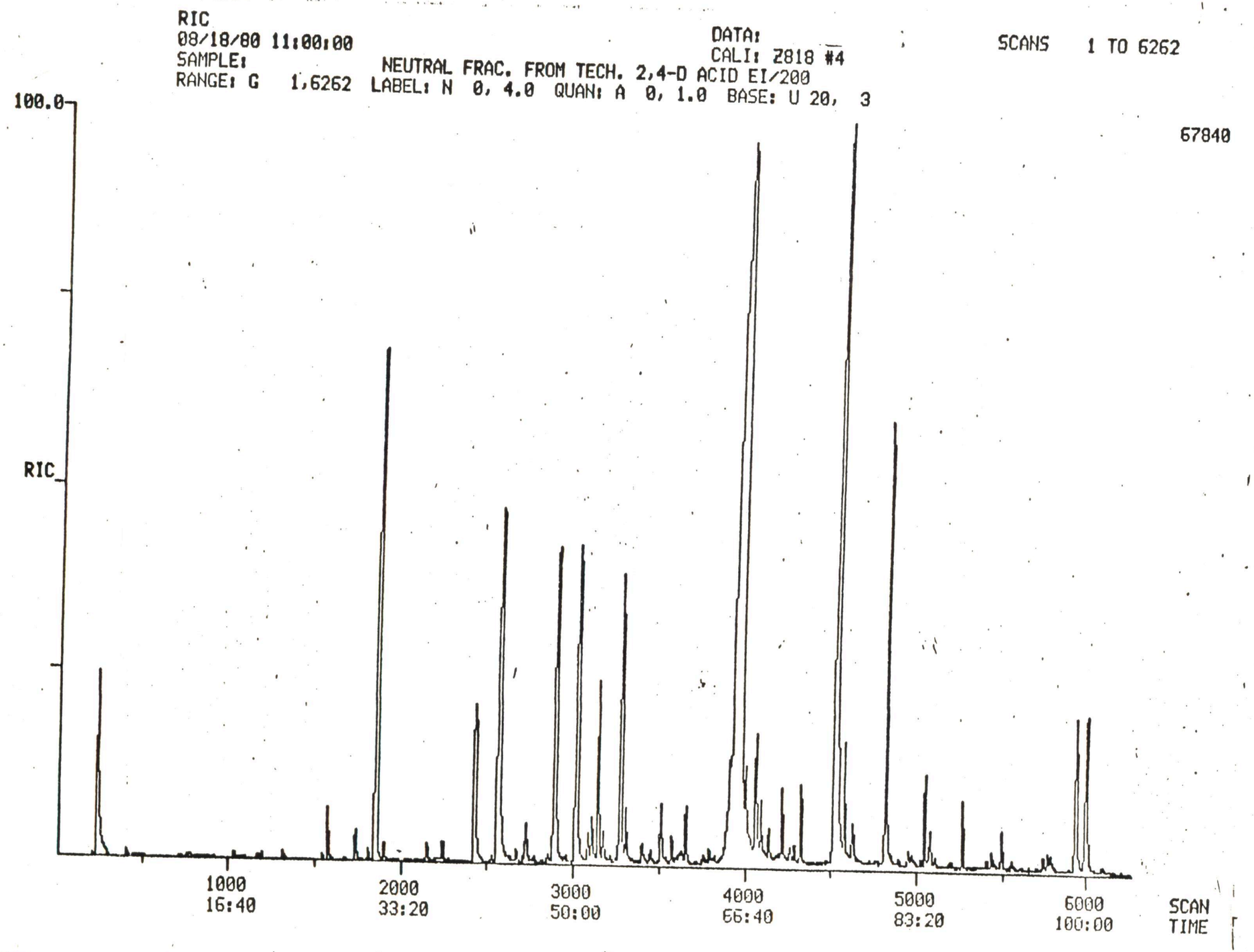
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DATA:
CALI: Z818 #4
SCANS 1 TO 6262
NEUTRAL FRAC. FROM TECH. 2,4-D ACID EI/200
LABEL: N 0, 4.0 QUAN: A 0, 1.0 BASE: U 20, 3
100.0
67840
RIC
1000
16:40
2000
33:20
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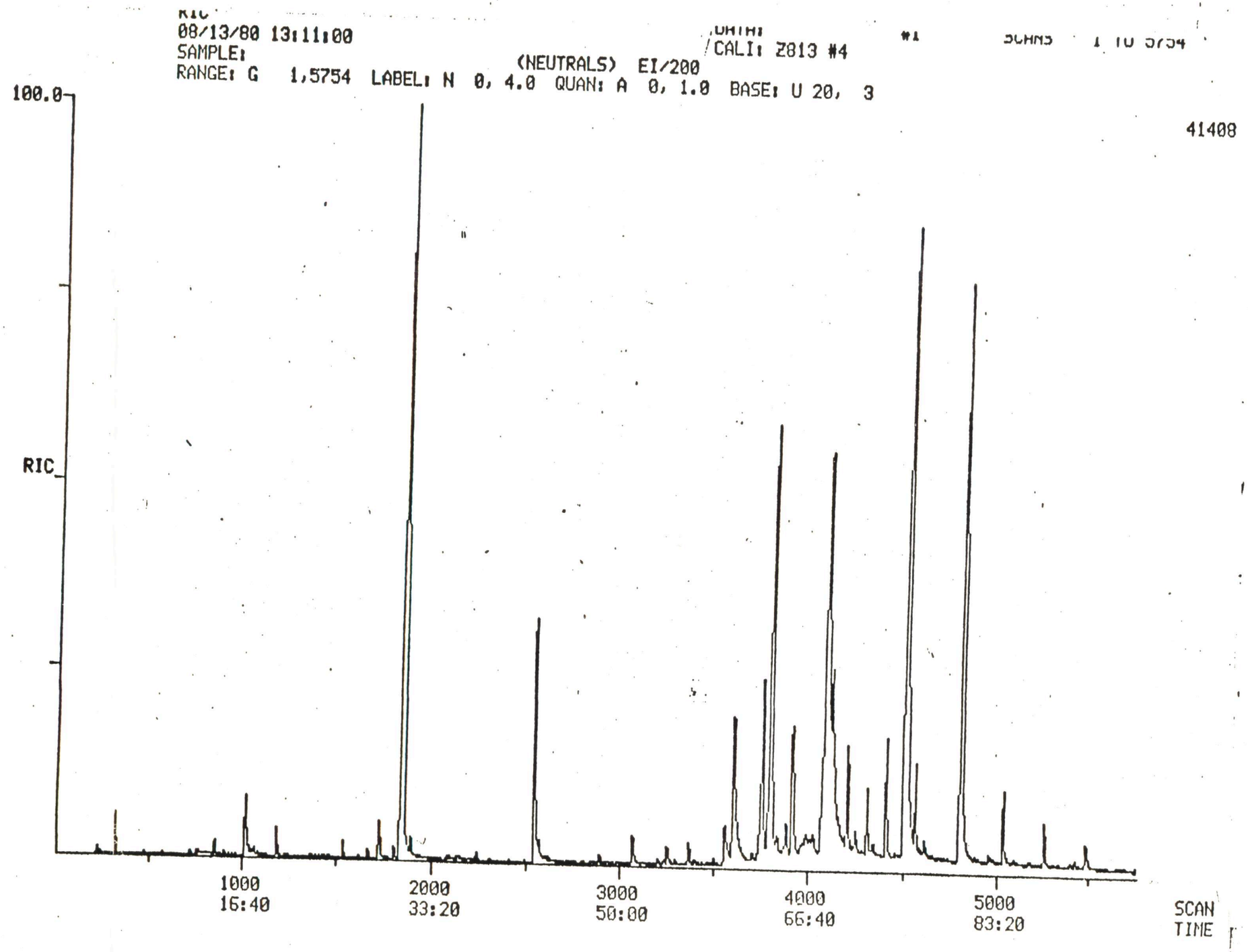
66:40
5000
83:20
6000
100:00
SCAN
TIME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08/13/80 13:11:00
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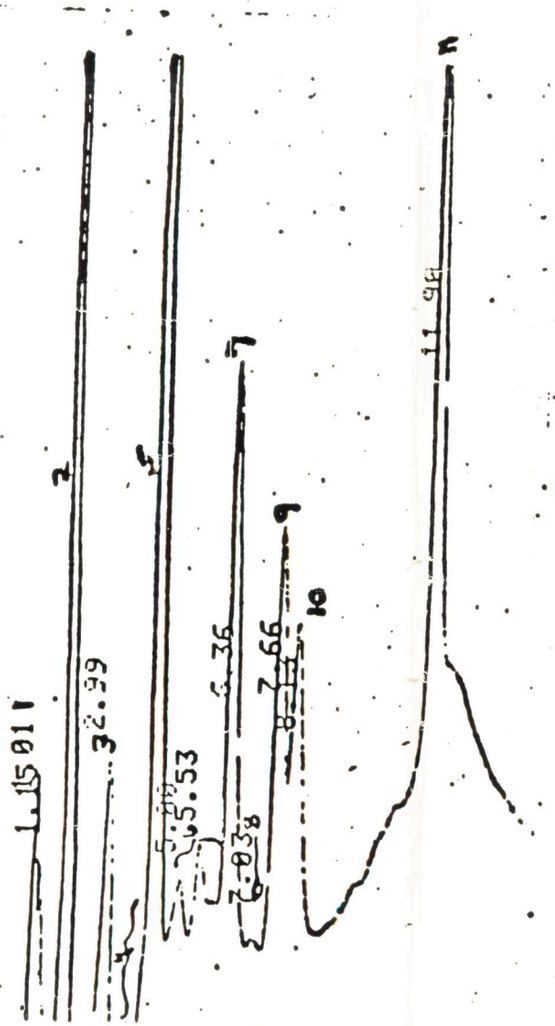
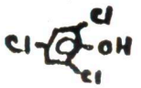
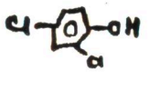
(NEUTRALS) EI/200
CALI: Z813 #4
RANGE: G 1,5754 LABEL: N 0, 4.0 QUAN: A 0, 1.0 BASE: U 20, 3
41408
100.0
RIC
1000
16:40
2000
33:20
3000
50:00
4000
66:40
5000
83:20
SCAN
TIME

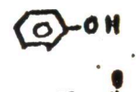
HPLC of a phenol mixture from a butoxyethyl ester

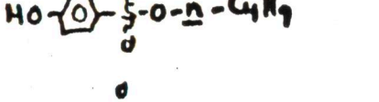
* silica column, 1CV : cyclohexane + 0.1% HOAc
2CV : 4:1 (v:v) DCM : THF
10% → 60% / 10 min, 3 mL/min

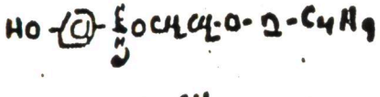


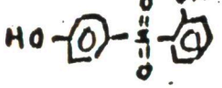
2. 

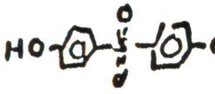
3. 

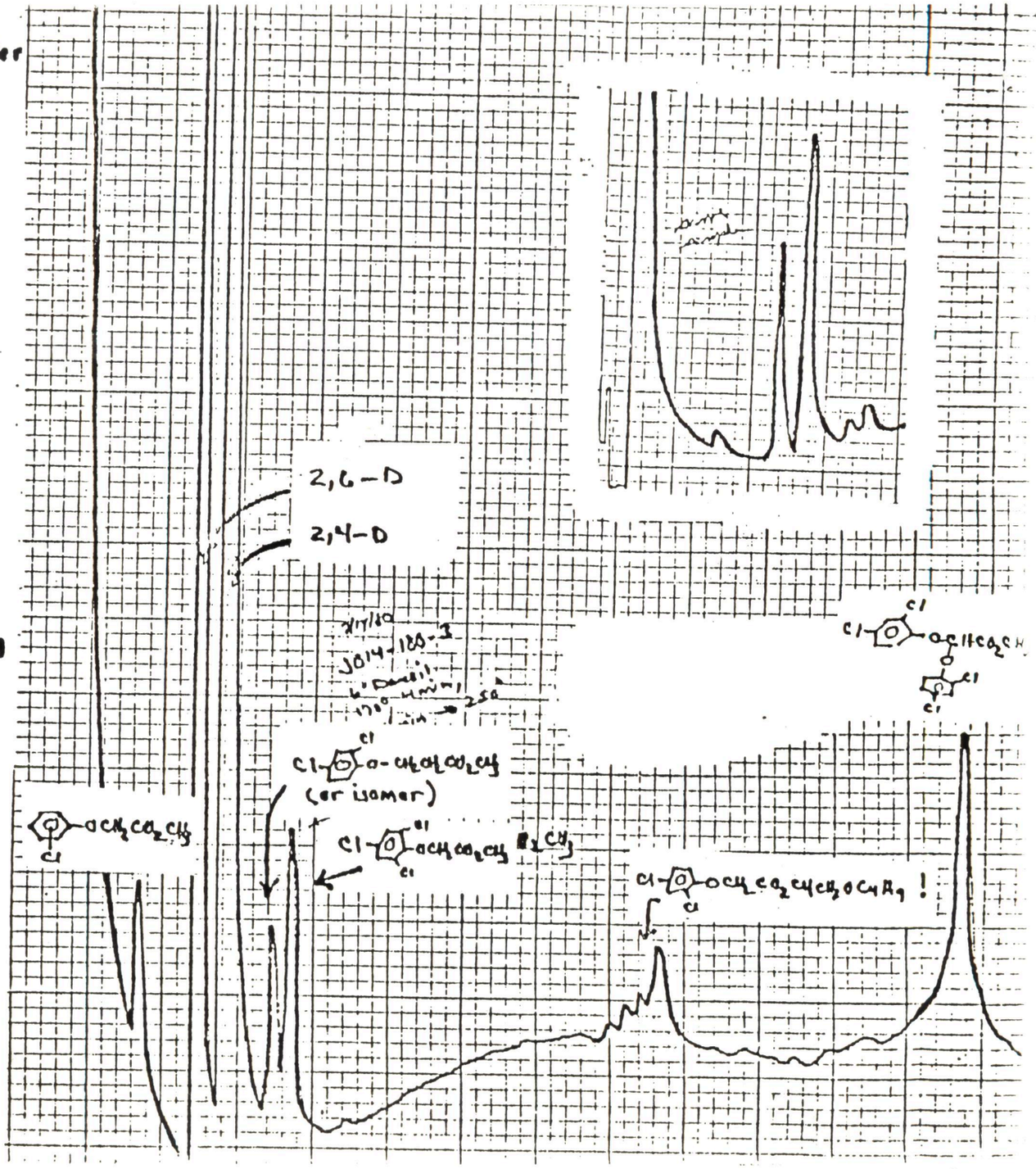
5. 

7. 

9. 

10. 

11. 



(Attachment 4)

MEMORANDUM OF CONFIDENTIALITY

This Agreement made and entered into by and among (Company 1), (Company 2), (Company 3), (Company 4), (Company 5), and (Company 6) (hereinafter referred to as "Technical Committee representatives"),

WITNESSETH:

WHEREAS, by Order and Notice dated August 29, 1980 the United States Environmental Protection Agency (hereinafter EPA), Office of Toxic Substances, under authority of § 3(c)(2)(B) of FIFRA required 2,4-D pesticide registrants, including the Task Force members, to develop and submit eight types of additional data on 2,4-D (hereinafter "additional data") under a specified schedule; and

WHEREAS, § 3(c)(2)(B)(ii) of FIFRA provides, in part, that "[t]wo or more registrants may agree to develop jointly, or to share in the cost of developing, such [additional] data if they agree and advise the Administrator of their intent within ninety days after notification . . . .";

WHEREAS, the Task Force members hold registrations for manufacturing-use products of 2,4-D under the Federal Insecticide, Fungicide and Rodenticide Act, as amended (hereinafter FIFRA) and, in the furtherance of their business, desire to preserve their registrations for such products under FIFRA.

WHEREAS, members of the Task Force have entered into a memorandum of Understanding dated _____ , 1980; and

WHEREAS, the Task Force authorized the formation of a Technical Committee consisting of a representative from each member of the Task Force and whose purpose is the general supervision and management of the testing work to develop the additional data; and

WHEREAS, during the course of its activities members of the Technical Committee will likely to become familiar with secret or confidential information of other members of the Task Force in order to complete the testing necessary to develop the additional data and to meet the objections of the Task Force;

NOW THEREFORE, in consideration of the mutal understanding described herein, the parties do hereby agree and contract, one with another, as follows:

1. Definition

Confidential information - as used herein, shall include but not necessarily be limited to, any of the following: types and kinds of raw materials used by Task Force members in the manufacture of 2,4-D formulae and compounding instructions covering the manufacture of the member's 2,4-D products; ingredient and/or analytical statements; description of manufacturing processes; and other information of a confidential nature concerning the manufacture of 2,4-D products, which is required to be maintained as such for the continued success of the members and their business. All confidential information exchanged among the undersigned shall be clearly marked as such.

2. Confidentialitv

(a) The undersigned members of the 2,4-D Technical Committee do hereby agree that it may become necessary during the course of the Committee's activities to exchange confidential information among themselves in order to make determinations and accomplish the testing objectives of the Task Force. The undersigned members of the 2,4-D Technical Committee do hereby agree that any exchange of confidential information will be solely for the purpose of making necessary determinations concerning the testing objectives of the Task Force. Such determinations shall include, but not necessarily limited to, determinations of ingredients of the members' 2,4-D products in order to arrive at a representative sample/s to be tested by the Task Force.

(b) The undersigned members of the Technical Committee do hereby agree that, in the event that any confidential information is exchanged by Committee members for the purposes described in paragraph 2(a), they will not disclose to anyone other than the undersigned any secret or confidential information obtained by or entrusted to them as a consequence of such exchange of information. Nor shall the undersigned duplicate in any manner such confidential information. The undersigned hereby agree that any confidential information obtained as a consequence of this Agreement shall be used solely for the purposes described in paragraph 2(a) above.

(c) The undersigned members of the Technical Committee do hereby agree that in the event of the termination of any member's employment prior to the conclusion of the Technical Committee's activities, that such member shall surrender to the owner all confidential information.

(d) The undersign hereby agree that at the conclusion of the activities of the Technical Committee or upon its dissolution by the Task Force, each member of the Task Force shall return to the owner all confidential information obtained as a consequence of this Agreement.

3 Arbitration

All disputes shall be submitted by the parties to arbitration under the auspices of the American Arbitration Association in accordance with its rules.

In witness whereof, the parties have duly executed this Agreement:

(Attachment 5)

Handout 5

PROTOCOL FOR A CHRONIC FEEDING-ONCOGENICITY STUDY ON 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ADMINISTERED IN THE DIET TO CDF FISCHER 344 RATS

I. Objective: The objective of this study is to evaluate the chronic toxicity and oncogenic potential of 2,4-D when administered to rats in the diet for two years. This study will be conducted essentially in accordance with Section 163.83-1 of the Proposed Guidelines for Registering Pesticides in the U.S.; Hazard Evaluation: Humans and Domestic Animals. (FR, Vol. 43, Aug. 22, 1978) and Good Laboratory Practice Regulations, FDA (FR, Vol. 43, Dec. 22, 1978 part II).

II. Sponsor:

III. Proposed; Starting Date: Completion Date:

Date of Final Report:

IV. Test Material:

A. Analysis: The test materials will be provided by the sponsor. The sponsor will be responsible for analyzing the test material and providing these data in an addendum to the final study report.

B. Stability: 2,4-D is very stable when stored in tightly closed container at ambient room temperature.

C. Storage Conditions: The test material shall be stored in the tightly sealed original containers at ambient room temperature during the duration of the study. Any unused test material will be returned to the sponsor.

At initiation and every six months during the study, a 10 gram sample of the test material will be removed from the original container in use at that time and returned to the sponsor.

VI. Test Animals: CDF Fischer 344 rats (Charles River Breeding Laboratory, Portage, MI) 3-5 weeks of age. Extra animals will be ordered to insure sufficient animals of acceptable health and weight are available to conduct the study as designed. This strain of rat has been selected because of its suitability for toxicity and oncogenicity testing, availability of historical background data and reliability of the supplier.

Upon arrival at the laboratory, the rats will be examined for health status by a qualified professional who will so indicate, sign and date the appropriate laboratory record book. The animals will be acclimated to the laboratory environment at least 7 days prior to weighing and random assignment to test levels.

Randomization will be accomplished by an acceptable procedure. Animals found to be outliers on the basis of their body weight (outliers identified by sequential method described by Grubbs, 1969) will be discarded from the study. The animals from the extremes of the distribution will be identified and removed from the test population until only the number of rats required for the basic study (including interim kills) remains. The animals remaining will be ranked by body weight, grouped (group size equal to the number of dose levels/sex) and one animal from each group randomly assigned to each dose level; this procedure increases the probability of uniform group means and standard deviations at the start of the study. Animals removed from the test population prior to group assignments will be maintained in the study room for two weeks and serve as the source for test animals requiring replacement for health reasons within this interval. After this period, animals are not to be replaced and the extra rats removed from the study room and used as stock animals or discarded.

All animals will be individually identified by a numbered ear tag. In the event that an ear tag becomes dislodged during the study, it is to be replaced with one having the same number and noted in the study file.

Animals will be housed individually in wire mesh cages. Cages are to be washed in accordance with good husbandry practices at least once per week. The transfer will include a clean rack, food crock, water supply and absorbent material. The study is to be conducted in a room designed for controlled humidity (40-60%), temperature (22 ± 2°C), a 12-hour photo cycle and an air change approximately every five minutes. Food (Purina's Certified Rodent Chow #5002) and water will be available ad libitum throughout the study.

VII. Test Diet: Analysis on Purina's Certified Rodent Chow® #5002 will be performed by the Ralston Purina Company to confirm that the diet provides adequate nutrition, and to quantitate the levels of selected chemical contaminants. Certification that each lot of feed meets label specifications will be obtained. A sample of each lot of the Certified Rodent Chow will be retained for possible additional analysis if deemed necessary by the study director. Analysis of tap water will be performed according to the Standard Operating Procedure of the testing laboratory.

Data generated in the laboratory of The Dow Chemical Company have shown 2,4-D to be stable in the diet for at least 1 month, consequently, a premix may be prepared twice monthly to prepare the diets for the respective dose levels. The test diets will be prepared weekly during the first three months of the study and monthly thereafter for the remainder of the study. The 2,4-D/Purina Certified Rodent Chow 5002 premix will be serially diluted with untreated chow to obtain the concentration of 2,4-D required for the respective experimental groups. The concentration of 2,4-D in

the diet will be adjusted weekly during the first three months of the study and monthly thereafter to maintain the designated dose levels on a mg/kg/day basis.

Core samples of mixed diets at each dose level will be analyzed at least 8 times during the study to confirm the concentration of 2,4-D in the diets. Reference diet samples (premix and 1/sex/dose level) will be retained every 3 months. A sample of Purina's Certified Rodent Chow 5002 (same lot number as reference diet samples) used to formulate the diets will also be retained quarterly. All diet mix samples will be discarded approximately 1 year after issuance of the final report.

VIII. Study Design:

A. The design for this tudy is indicated in Tables A and
Male and female weanling CDF Fischer 344 rats from sin
populations with weight ranges of 95-100 grams (males) a
85-120 grams (females) will be randomly assigned 60/sex
control and experimental groups for the basic study.
these, ten/sex/control and experimental groups will
randomly selected for the 12 month (± 1 month) inte
sacrifice.

TABLE A
TEST MATERIAL: 2,4-D
TWO-YEAR CHRONIC TOXICITY-ONCOGENICITY STUDY - CDF FISCHER 344 RA
STUDY DESIGN

Dose Level (mg/kg/day)	Two-Year Study No. of Rats/ sex/level	One-Year Interim No. of Rats/ sex/level	Total No. of Rat
	50	10	120
	50	10	120
	50	10	120
	50	10	120
			480

Parameter (No. rats/sex/group)	6, 12[a], 18 Month	Termination[b]
Hematology(10)	0, top dose	prior to terminatic all levels if indi- cated
Urinalysis (10)	0, top dose	prior to terminatic all levels if indi- cated
Clinical Chemistry (10)	all rats/all levels	all rats/all levels
Necropsy	10/sex, all levels[a]	all survivors
Histopathology	10/sex, 0, top dose[a], others if indicated by results obtained on the top dose animals	all rats/all levels

[a]Refers to animals designated for the one-year interim kill.

[b]Twenty-four months unless unforeseen developments dictate otherwise.

B. Observations: All animals will be observed twice daily for signs of toxicity or changes in demeanor according to the Standard Operating Procedures of the laboratory. All animals will be examined at least monthly after the first month for palpable masses and any findings recorded as to size, location and subsequent growth.

1. Body Weight: Individual body weights will be recorded weekly for the first 13 weeks and at least monthly thereafter. A subgroup consisting of 20 animals/sex/-group randomly selected from the basic study animals will be utilized to generate body weight data required to calculate the diet mixing instructions for all rats on the respective test levels. If a death is recorded in the subgroup, the animal(s) in the next cage in the random number sequence will be included for subsequent calculations; thus, the N number of participants will be a minimum of 20.

2. Food Consumption: Food consumption of the animals in the "subgroup" animals will be determined on a weekly basis during the first three months and over a period of one week each month thereafter with an attempt being made to record the measurements the same week each month. The food consumption and bodyweight data from the "subgroup" (considered to be representative for all animals on test) of the basic study for each test level will be used to adjust the concentration of 2,4-D in the diet to maintain the dose levels on a mg/kg/day basis for all animals.

3. Mortality: Rats dying during the course of the study will be refrigerated and necropsied as soon as possible. Moribund animals will be sacrificed when death appears imminent and necropsied. Necropsied animals will be examined externally and internally and any observations recorded. The routine tissues to be collected at interim necropsy are the same as at terminal necropsy.

4. Clinical Evaluations

   a. Hematology evaluation will be conducted on 10 rats/sex/dose at 6, 12, 18 months and at sacrifice. Hemotologic evaluations will include; hematocrit, hemoglobin, erythrocyte count, total and differential loucocyte count, platelet count and if anemia is present, reticulocyte count.

   b. Urinalysis will be performed concurrently with hematology and on the same animals. Tests will include semiquantitative estimate of pH, specific quantity, bilirubin, glucose, protein, ketones and urobilogen.

5. Blood chemistry determinations will be conducted on 10 rats/sex/dose at 6, 12, 18 months and at termination. Determinations will include; calcium, potassium, serum-lactic dehydrogenase, SGPT, SGOT, BUN, alkaline phosphotase activity, total bilirubin, total protein, albumin, globulin, glucose and total thyroxine (T_4).

6. Pathology: At 12 months 10 animals/sex/dose will be fasted overnight, weighed, sacrificed by decapitation following anethesia and clamping of the trachea, and subjected to a complete gross necropsy examination. Weights of brain, heart, liver, kidney and testes will be recorded and the organ weight to final body weight ratios calculated. The tissues listed in Table B will be collected and preserved in phosphate buffered 10% formalin.

At the end of the study, all rats will be fasted overnight, weighed, killed by decapitation following anesthesia and clamping of the trachea, and subjected to a complete gross necropsy examination. They will be examined externally and internally by a veterinary pathologist and any observations will be recorded. Blood for serum may be collected from a minimum of 10/sex/dose from the severed cervical blood vessels at the time of decapitation. Weights of the brain, heart, liver, kidney and testes will be recorded and the organ weight to final body weight ratios calculated. The tissues listed in Table B will be collected from all rats and will be preserved in phosphate-buffered 10% formalin. Tissues for histopathological examination (Table B) will be imbedded in paraffin, sectioned ($\sim$5-6 μm) and stained with hematoxylin and eosin.

Ophthalmologic Examination. At necropsy, the eyes of all animals will be examined in situ immediately after decapitation by means of a glass microscope slide technique and fluorescent illumination.

IX. Statistical Evaluation: Clinical chemistry, hematology (excluding white cell differential count), urinary specific gravity, food and water consumption, organ weights, body weights and organ to fasted body weight ratio data will be evaluated by appropriate statistical methods.

Statistical evaluation of survival indices will be by the Wilcoxon t-test as modified by Haserman and Hoel (1974). Analysis of tumor frequencies will be done using the Fischer Exact Probability Test (Siegel, 1956). If non-treatment related differential mortality occurs, tumor data will be analyzed by life table technique if appropriate. The levels of significant for statistical test are as follows:

Analysis of Variance	$p<0.10$
t-test Comparison	$p<0.05$[a] two-sided
Outlier Determination	$p<0.02$ two-sided

[a]Although statistical analysis of data may be performed at other p values to aid in the interpretation of trends in the analysis (i.e. $p<0.1$), only values that are statistically significant at $p<0.05$ will be indicated in the report tables.

In certain instances, further statistical methods may be used if appropriate.

X. Reports: A letter report will be submitted every three months after study initiation by sex and dose on; mortality, mean body weight, food consumption and palpable masses. A summary report will be issued after the 12 month interim sacrifice which will include a summary of the hematology, urinalysis, clinical shemistries and gross necropsy findings.

A final report will be issued within 12 months of the terminal sacrifice.

TABLE B

TISSUES FOR HISTOPATHOLOGICAL EXAMINATION

all gross lesions	coagulating gland	parathyroid gland
liver (2 lobes)		
heart	prostate	trachea
spleen	urinary bladder	skin
pituitary	ureter	mammary gland
pancreas	urethra	preputial or clitoral gland
bone	ovary	eye
bone marrow	oviduct	tongue
adrenal	uterus	oral cavity
kidneys	cervix	nasal turbinates
stomach	vagina	mesenteric tissue
small intestine	lungs	lacrimal gland
cecum	mainstem bronchi	larynx
	skeletal muscle	
large intestine	salivary gland	zymbal gland
cervical lymph node	thymus	forebrain
mediastinal lymph node	mediastinal tissue	mid brain
mesenteric lymph node	aorta	hind brain
other miscellaneous lymph nodes	esophagus	spinal chord (2 levels)
testicle	thyroid gland	sciatic nerve
epidiymis	seminal vesicle	peripheral nerve